

WHAT IS CLAIMED IS:

1. A method for determining activity of an enzyme ~~the production of an amino acid from a target 2-ketoacid~~ comprising:
contacting the enzyme with a composition comprising a target compound ~~creating a mutated enzyme that catalyzes the reductive amination or transamination of the target 2-ketoacid~~; and
thereafter determining whether there is a change in the pH of the composition ~~providing the mutated enzyme in a reaction mixture comprising the target 2-ketoacid under conditions sufficient to permit the formation of the amino acid to thereby produce the amino acid~~.
2. The method of claim 1, wherein the determining step ~~comprises detecting an optical change in the composition~~ ~~mutated enzyme catalyzes the reductive amination of the target 2-ketoacid~~.
3. The method of claim 1, wherein the composition further ~~comprises a pH indicator, wherein the determining step comprises detecting the pH change using the pH indicator~~ ~~2, wherein the mutated enzyme is an amino acid dehydrogenase~~.
4. The method of claim 3, wherein the pH indicator causes the composition to change color if there is a change in the pH of the composition ~~amino acid dehydrogenase is a leucine dehydrogenase~~.
5. The method of claim 3, wherein the target compound is ethyl 4 chloro 3 ketobutyrate and the pH indicator is cresol red ~~amino acid dehydrogenase is a phenylalanine dehydrogenase~~.
6. The method of claim 3, wherein the pH indicator is selected from the group consisting of cresol red, m cresol purple, bromothymol blue, bromophenol red, bromophenol blue, phenol red, and phenolphthalein.
6. The method of claim 2, further comprising providing an existing enzyme that catalyzes the reductive amination of a 2-ketoacid, wherein the mutated enzyme is created by mutating the existing enzyme, and further wherein the mutated enzyme catalyzes the reductive amination of the target 2-ketoacid at a greater rate than the existing enzyme.
7. The method of claim 1, wherein the composition further ~~comprises a cofactor~~ ~~mutated enzyme catalyzes the transamination of the target 2-ketoacid~~

8. The method of claim 7, wherein the ~~cofactor~~mutated enzyme is selected from the group consisting of ~~nicotinamide adenine dinucleotide (NAD⁺)~~, a reduced form of ~~nicotinamide adenine dinucleotide (NADH)~~, ~~nicotinamide adenine dinucleotide phosphate (NADP⁺)~~, a reduced form of ~~nicotinamide adenine dinucleotide phosphate (NADPH)~~, and derivatives thereof aspartic-glutamic transaminases, aromatic amino acid transaminases, and branched-chain amino acid transaminases.

9. The method of claim 7, wherein the cofactor is a ~~pyridine nucleotide or a flavin~~further comprising providing an existing enzyme that catalyzes the transamination of a 2-ketoacid, wherein the mutated enzyme is created by mutating the existing enzyme, and further wherein the mutated enzyme catalyzes the transamination of the target 2-ketoacid at a greater rate than the existing enzyme.

10. The method of claim 1, wherein the ~~enzyme~~ is an ~~oxidoreductase~~reaction mixture further comprises ammonia or a salt thereof.

11. The method of claim 10, wherein the ~~oxidoreductase~~ is at least one of a reductase, an oxidase, a dehydrogenase, and a ketoreductase, wherein the reaction mixture further comprises a nicotinamide cofactor.

12. The method of claim 10, wherein the ~~oxidoreductase~~ is selected from the group consisting of ~~alcohol dehydrogenases, carbonyl reductases, aldehyde dehydrogenases, amino acid dehydrogenases, amine oxidases, disulfide reductases, enoate reductases, and mixed function oxidases~~11, wherein the nicotinamide cofactor is recycled.

13. The method of claim 1, wherein the ~~target compound~~ is converted into an enantiomerically enriched chiral compound by the enzymeamino acid is chiral.

14. The method of claim 1, wherein the ~~target compound~~2-ketoacid is selected from the group consisting of ~~aldehydes, ketones, disulfides, thiols, ketoacids, amines, amino acids, alcohols, alkenes, alkanes, and compounds that are converted to aldehydes, ketones, disulfides, thiols, ketoacids, amines, amino acids, alcohols, alkenes, and alkanes~~3,3-dimethyl-2-ketobutyrate, 3-(2-naphthyl)pyruvate, 3-(1-naphthyl)pyruvate, and 4-(methylphosphinyl)-2-ketobutyrate.

15. The method of claim 1, wherein the composition further comprises a buffer, mutated enzyme is created by:
providing an existing enzyme;
mutating the existing enzyme to produce the mutated enzyme;
determining the activity of the mutated enzyme on the target
2-ketoacid by contacting the mutated enzyme with a composition
comprising the target 2-ketoacid and thereafter determining
whether there is a change in the pH of the composition; and
determining whether the mutated enzyme catalyzes the
reductive amination or transamination of the target 2-ketoacid at
a greater rate than the existing enzyme.

16. The method of claim 1, wherein the enzyme is present in a material to be screened that is in the form of a solution, a suspension, or a dried mixture¹⁵, wherein the determining step comprises detecting an optical change in the composition.

17. The method of claim 1, wherein the enzyme is present in a material to be screened, wherein the material to be screened is selected from the group consisting of cell lysates, a mixtures of cells, and cell extracts, composition further comprises a pH indicator, wherein the determining step comprises detecting the pH change using the pH indicator.

18. A method for determining activity the production of an oxidoreductase amine from a target ketone comprising:

contacting the oxidoreductase with a composition comprising a target compound, a cofactor, and a pH indicator;
allowing the oxidoreductase to act on the target compound for a sufficient time to catalyze a reaction; and
thereafter detecting a change in the pH of the composition using the pH indicator.
creating a mutated enzyme that catalyzes the reductive amination or transamination of the target ketone; and
providing the mutated enzyme in a reaction mixture comprising the target ketone under conditions sufficient to permit the formation of the amine to thereby produce the amine.

19. The method of claim 18, wherein the pH indicator causes an optical change in the composition if there is a change in the pH of the composition mutated enzyme catalyzes the reductive amination of the target ketone.

20. The method of claim 18, wherein the pH indicator is selected from the group consisting of cresol red, m cresol purple, bromothymol blue, bromophenol red, bromophenol blue,

phenol red, and phenolphthalein¹⁹, wherein the mutated enzyme is an amino acid dehydrogenase.

21. The method of claim 18, wherein the cofactor is selected from the group consisting of nicotinamide adenine dinucleotide (NAD⁺), a reduced form of nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADP⁺), a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), and derivatives thereof.

21. The method of claim 19, further comprising providing an existing enzyme that catalyzes the reductive amination of a ketone, wherein the mutated enzyme is created by mutating the existing enzyme, and further wherein the mutated enzyme catalyzes the reductive amination of the target ketone at a greater rate than the existing enzyme.

22. The method of claim 18, wherein the cofactor is a pyridine nucleotide or a flavinmutated enzyme catalyzes the transamination of the target ketone.

23. The method of claim 18, wherein the oxidoreductase is at least one of a reductase, an oxidase, a dehydrogenase, and a ketoreductase²², wherein the mutated enzyme is selected from the group consisting of aspartic-glutamic transaminases, aromatic amino acid transaminases, and branched-chain amino acid transaminases.

24. The method of claim 18, wherein the oxidoreductase is selected from the group consisting of alcohol dehydrogenases, carbonyl reductases, aldehyde dehydrogenases, amino acid dehydrogenases, amine oxidases, disulfide reductases, enoate reductases, and mixed function oxidases.

24. The method of claim 22, further comprising providing an existing enzyme that catalyzes the transamination of a ketone, wherein the mutated enzyme is created by mutating the existing enzyme, and further wherein the mutated enzyme catalyzes the transamination of the target ketone at a greater rate than the existing enzyme.

25. The method of claim 18, wherein the target compound is stereoselectively converted to a chiral compound by the oxidoreductase. mutated enzyme is created by:

providing an existing enzyme;

mutating the existing enzyme to produce the mutated enzyme;

determining the activity of the mutated enzyme on the target ketone by contacting the mutated enzyme with a composition comprising the target ketone and thereafter determining whether there is a change in the pH of the composition; and

determining whether the mutated enzyme catalyzes the reductive amination or transamination of the target ketone at a greater rate than the existing enzyme.

26. The method of claim 18, wherein the target compound is selected from the group consisting of aldehydes, ketones, disulfides, thiols, ketoacids, amines, amino acids, alcohols, alkenes, alkanes, and compounds that are converted to aldehydes, ketones, disulfides, thiols, ketoacids, amines, amino acids, alcohols, alkenes, and alkanes.

26. A method for the production of an alcohol from a target ketone comprising:

creating a mutated enzyme that catalyzes the reduction of the target ketone; and

providing the mutated enzyme in a reaction mixture comprising the target ketone under conditions sufficient to permit the formation of the alcohol to thereby produce the alcohol.

27. The method of claim 18, wherein the composition further comprises a buffer²⁶, wherein the mutated enzyme is selected from the group consisting of alcohol dehydrogenases, ketoreductases, and carbonyl reductases.

28. The method of claim 18,²⁶, wherein the oxidoreductase is present in a material to be screened that is in the form of a solution, a suspension, or a dried mixture.mutated enzyme is the alcohol dehydrogenase YPR1.

29. The method of claim 18, wherein the oxidoreductase is present in a material to be screened, wherein the material to be screened is selected from the group consisting of cell lysates, a mixtures of cells, and cell extracts.

29. The method of claim 26, further comprising providing an existing enzyme that catalyzes the reduction of a ketone, wherein the mutated enzyme is created by mutating the existing enzyme, and further wherein the mutated enzyme catalyzes the reduction of the target ketone at a greater rate than the existing enzyme.

30. The method of claim 18, wherein the reaction is at least one of an oxidation reaction, a reduction reaction, a dehydrogenation reaction, a condensation reaction, and an electron transfer reaction.²⁶, wherein the mutated enzyme is created by:

31. A kit for screening providing an existing enzyme-activity, the kit comprising:

(a) a material to be screened containing an enzyme;

- (b) a container; and
- (c) a pH indicator.

32. The kit of claim 31, wherein the enzyme is an oxidoreductase.

33. The kit of claim 31, wherein the kit further comprises a cofactor.

mutating the existing enzyme to produce the mutated enzyme;

34. The kit of claim 31, wherein the pH indicator causes an optical change in the composition if determining the activity of the mutated enzyme on the target ketone by contacting the mutated enzyme with a composition comprising the target ketone and thereafter determining whether there is a change in the pH of the composition; and

35. A kit for screening enzyme activity, the kit comprising:

- (a) a plurality of containers;
- (b) a material to be screened containing an enzyme in each container; and
- (c) a pH indicator.

36. The kit of claim 35, wherein each container contains a different material to be screened.

37. The kit of claim 35, wherein the pH indicator is present in each container.

38. The kit of claim 35, further comprising a cofactor.

39. The kit of claim 38, wherein the cofactor is present in each container.

40. A kit for screening oxidoreductase activity, the kit comprising:

- (a) a plurality of containers;
- (b) a material to be screened containing an oxidoreductase in each container;
- (c) a pH indicator; and
- (d) a cofactor.

41. The kit of claim 40, wherein the pH indicator is present in each container.

42. The kit of claim 40, wherein the cofactor is present in each container.

43. The kit of claim 40, wherein the pH indicator is a pH indicator that causes an optical change upon a pH change.

44. The kit of claim 40, wherein the pH indicator is selected from the group consisting of cresol red, m cresol purple, bromothymol blue, bromophenol red, bromophenol blue, phenol red, and phenolphthalein.

45. The kit of claim 40, wherein cofactor is selected from the group consisting of nicotinamide adenine dinucleotide (NAD⁺), a reduced form of nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate (NADP⁺), a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), and derivatives thereof.

46. The kit of claim 40, wherein the cofactor is a pyridine nucleotide or a flavin.

47. The kit of claim 40, wherein the oxidoreductase is at least one of a reductase, an oxidase, a dehydrogenase, and a ketoreductase.

48. The kit of claim 40, wherein the oxidoreductase is selected from the group consisting of an alcohol dehydrogenase, a carbonyl reductase, an aldehyde dehydrogenase, an amino acid dehydrogenase, an amine oxidase, a disulfide reductase, an enoate reductase, and a mixed function oxidase.

49. The kit of claim 40, further comprising a buffer. determining whether the mutated enzyme catalyzes the reduction of the target ketone at a greater rate than the existing enzyme.

50. The kit of claim 49, wherein the buffer is present in each container.

51. The kit of claim 40, wherein the material to be screened is selected from the group consisting of cell lysates, a mixtures of cells, and cell extracts.